Original Article

Telemetered common marmosets is useful for the assessment of electrocardiogram parameters changes induced by multiple cardiac ion channel inhibitors

Kenta Watanabe^{1,2}, Tadashi Tsubouchi¹, Toru Yamada¹, Eiichi Hinoi² and Izuru Miyawaki¹

¹Preclinical Research Unit, Sumitomo Dainippon Pharma Co., Ltd.,
3-1-98 Kasugade-naka, Konohana-ku, Osaka 554-0022, Japan

²Laboratory of Molecular Pharmacology, Division of Pharmaceutical Sciences,
Kanazawa University Graduate School, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan

(Received November 18, 2018; Accepted April 14, 2019)

ABSTRACT — The objective of this study is to assess the response of telemetered common marmosets to multiple cardiac ion channel inhibitors and to clarify the usefulness of this animal model in evaluating the effects of drug candidates on electrocardiogram (ECG). Six multiple cardiac ion channel inhibitors (sotalol, astemizole, flecainide, quinidine, verapamil and terfenadine) were orally administered to telemetered common marmosets and changes in QTc, PR interval and QRS duration were evaluated. Drugs plasma levels were determined to compare the sensitivity in common marmosets to that in humans. QTc prolongation was observed in the marmosets dosed with sotalol, astemizole, flecainide, quinidine, verapamil and terfenadine. PR prolongation was noted after flecainide and verapamil administration, and QRS widening occurred following treatment with flecainide and quinidine. Drugs plasma levels associated with ECG changes in marmosets were similar to those in humans, except for verapamil-induced QTc prolongation. Verapamil-induced change is suggested due to body temperature decrease. These results indicate that telemetered common marmoset is a useful animal for evaluation of the ECG effects of multiple cardiac ion channel inhibitors and the influence of body temperature change should be considered in the assessment.

Key words: Common marmosets, Arrhythmia, Electrocardiogram

INTRODUCTION

Drug-induced cardiac arrhythmia is considered as a critical toxicity issue in drug development. Especially, polymorphic ventricular tachycardia, called torsades de pointes (TdP), is life-threatening (Johnston *et al.*, 2013) and has been associated with the withdrawal of a number of prescribed drugs (Li and Ramos, 2017). Since it is known that drugs that induce TdP in humans, cause the prolongation of corrected QT interval (QTc) through the inhibition of human ether-a-go-go-related gene (hERG) channel (Redfern *et al.*, 2003), drug candidates *in vitro* hERG inhibition is commonly assessed in an early stage of drug development to screen out proarrhythmic compounds. In addition to hERG, other cardiac ion channels, such as voltage-dependent sodium channel type 1.5 (Nav1.5) and voltage-dependent calcium channel type 1.2 (Cav1.2), also contribute to proarrhythmic liability, suggesting that Nav1.5 inhibition results in PR

prolongation and QRS widening, and Cav1.2 inhibition causes PR prolongation and QTc shortening (Priest and McDermott, 2015; Bergenholm *et al.*, 2017; Isobe *et al.*, 2018). Although *in vitro* and *in silico* assay systems that use *in vitro* data of multiple cardiac ion channel inhibitory potential have been reported as challenges for TdP risk prediction (Kramer *et al.*, 2013; O'Hara *et al.*, 2011; Li *et al.*, 2019), *in vivo* studies are considered as the definitive approach. Telemetry study, using conscious beagle dogs or cynomolgus monkeys, is the golden standard for TdP risk assessment because the study allows comprehensive evaluation of the effects of test articles and their metabolites by continuous ECG monitoring. Because a lot of test article is needed for the telemetry study due to large body size of beagle dogs and cynomolgus monkeys, it is practically difficult to conduct telemetry studies using those animals in early stage of drug development.

The common marmoset is one of the “New World Monkeys” (Saito, 2015) and the test using the common

marmosets is expected to be conducted with small amount of test article because of their small body size. Therefore, a telemetry study using common marmoset is considered useful as a comprehensive TdP risk assessment, which can be conducted in early stage, if TdP risk is appropriately assessed in common marmosets as well as beagle dogs and cynomolgus monkeys. The possibility of the use of common marmosets in proarrhythmia risk assessment has been investigated. It has been reported that QTc prolongation induced by astemizole, sotalol or moxifloxacin was detected in common marmosets (Horii *et al.*, 2002; Tabo *et al.*, 2008), and the sensitivity of QTc prolongation induced by moxifloxacin in marmosets was similar to that in humans (Komatsu *et al.*, 2010). In addition, propranolol and nifedipine, non-QTc prolonging drugs, are reported not to prolong QTc in common marmosets (Tabo *et al.*, 2008). These reports demonstrate that hERG inhibition-related QTc prolongation can be detected in common marmosets with a sensitivity similar to humans.

Although common marmosets are known to be a useful animal for QTc assessment of hERG blockers, it has not been particularly investigated whether the changes in PR interval and QRS duration can be detected. Therefore, in this study, we assessed the effects of multiple cardiac ion channel inhibitors, such as sotalol, astemizole, flecainide, quinidine, verapamil and terfenadine, on PR interval, QRS duration and QTc, in common marmosets. In addition, the relationship between their plasma exposure level and changes in the ECG parameters were also evaluated.

MATERIALS AND METHODS

Animals

This study was approved by the Institutional Animal Care and Use Committee of Sumitomo Dainippon Pharma Co., Ltd. Eleven male common marmosets (CLEA Japan Inc., Tokyo, Japan) were used in the present study. Six male common marmosets aged 25 to 84 months and weighing 255 to 369 g were used for telemetry measurement, and 5 male common marmosets aged 22 to 85 months and weighing 296 to 373 g were used for blood sampling to toxicokinetics (TK). All common marmosets were housed individually in a standard environment (temperature: 25-29°C, relative humidity: 30-70%, lighting period: 7:00-19:00, air ventilation: 11-20 changes/hr). Solid food (CMS-1M, CLEA Japan Inc.) at 25 g was provided to each marmoset once daily, and water was available *ad libitum*. After completion of this study, all animals were cared for and used in another study.

Surgical procedure

The male common marmosets were inductively anesthetized with ketamine (10-20 mg/kg, i.m., Fujita pharmaceutical Co., Ltd., Tokyo, Japan) and xylazine (1-2 mg/kg, i.m., Bayer Yakuhin, Ltd., Osaka, Japan). Anesthesia was maintained by inhalation of isoflurane (1.5-2%, with air). Transmitters (HD-S11, Data Sciences International, St. Paul, MN, USA) were implanted into the peritoneal cavity, and ECG leads were placed subcutaneously in an approximate limb lead II configuration. A catheter for measurement of arterial blood pressure was inserted into the abdominal aorta. Penicillin (30,000 Unit, i.m., Kyoritsuseiyaku Corporation, Tokyo, Japan) were dosed on the day before the surgery day, and penicillin (15,000 Unit, i.m., Kyoritsuseiyaku Corporation) and meloxicam (1 mg/kg, i.m., Boehringer Ingelheim Pharma GmbH, Ingelheim am Rhein, Germany) were given to the marmosets before surgery and up to 5 days after surgery. The animals' recovery period was set as over 4 weeks from surgery.

Telemetry measurement

Three male animals per test article were orally dosed with the vehicle or the test article in a dose-escalation manner with an interval of at least 7 days between doses. General condition and ECG parameters were confirmed to return to basal level before dose level increase. The test articles were suspended in 0.5% methylcellulose solution (vehicle), and were dosed at around 10:00 with a dosing volume of 5 mL/kg. Arterial blood pressure, body temperature and ECG signals were continuously recorded from approximately 2 hr before dosing to approximately 25 hr after dosing using Dataquest ART data acquisition system (Data Sciences International), and ECG signals were recorded at 1,000 Hz. Animals health condition was continuously monitored using a video monitoring system. Test articles were dosed as following order: sotalol, flecainide, quinidine, astemizole, verapamil, terfenadine. Vehicle control was dosed every 1 week before the administration of the low dose of each test article. Dose levels and time points for ECG analysis and TK blood sampling of each test article are summarized in Table 1.

ECG parameters analysis

Telemetry data were analyzed using NOTOCORD-hem data analysis software (Notocord Systems SAS, Croissy Sur Seine, France). Fifteen ECG waveforms were analyzed manually at each time point and RR, PR and QT intervals, and QRS duration were determined. QTc was calculated using the following formula (Holzgrefe *et al.*, 2007):

Table 1. Test articles dose levels and time points of ECG parameters and TK measurements.

Test article	Dose level (mg/kg)	Time points (hr, post-dose)	
		ECG measurement	TK
Sotalol	5, 15	1, 2, 4, 8, 24	1, 2, 4, 8
Astemizole	3, 10, 30	1, 2, 4, 6, 24	1, 2, 4, 6
Flecainide	5, 15, 45	1, 2, 4, 6, 24	1, 2, 4
Quinidine	2, 10, 50	1, 2, 4, 6, 24	1, 2, 4, 6
Verapamil	15, 50, 150	1, 2, 4, 6, 24	1, 2, 4, 6
Terfenadine	30, 100, 300	1, 2, 4, 6, 24	1, 2, 4, 6

$$\log(QT_c) = \log(QT) - \beta(\log(RR) - \log(RR_{ref}))$$

where, QT and RR are the values obtained at each time point expressed in milli-second (msec), and RR_{ref} is the reference RR set as 400 msec, which is the prevalent value at rest for common marmosets (Honda *et al.*, 2010). To determine the β value for each animal, 24 hr telemetry ECG data were recorded without test article dosing and QT and RR intervals were averaged every 15 min for each animal. QT-RR relationship for each individual animal was fitted with a linear regression and the β value for each animal was determined as the slope of the equation. The β values of 6 common marmosets used for telemetry measurement in the present study were 0.454-0.634. In this study, arterial blood pressure was measured just to monitor whole body condition.

Dose levels and rationale for the doses of each compound

dl-sotalol hydrochloride (sotalol): Tabo *et al.* (2008) reported that oral administration of dl-sotalol hydrochloride at 15 mg/kg prolonged QTc by approximately 100 msec in conscious common marmosets. Therefore, the high dose was set at 15 mg/kg (as sotalol) as a dose at which marked QTc prolongation is considered to be observed. To investigate dose-relationship, 5 mg/kg (as sotalol) was set as the low dose with a common ratio of 3.

Astemizole: Tabo *et al.* (2008) reported that oral administration of astemizole at 10 and 30 mg/kg prolonged QTc by approximately 50 and 100 msec, respectively, in conscious common marmosets. Therefore, the high dose and the middle dose were set at 30 and 10 mg/kg as a dose at which QTc prolongation is considered to be observed. To investigate dose-relationship, 3 mg/kg was set as the low dose with a common ratio of approximately 3.

Flecainide acetate (flecainide): Heath *et al.* (2011) reported that oral administration of flecainide at 45 mg/kg (as flecainide) induced significant QRS widening in con-

scious rats. Therefore, the high dose was set at 45 mg/kg (as flecainide) as a dose at which significant QRS widening is considered to be observed if pharmacokinetics (PK) after oral administration to common marmosets is similar with that in rats. To investigate dose-relationship, 15 and 5 mg/kg (as flecainide) were set as the middle and the low dose, respectively, with a common ratio of 3.

Quinidine sulfate dihydrate (quinidine): Ando *et al.* (2005a) reported that oral administration of quinidine at 10 and 50 mg/kg prolonged QTc by 9% and 15% compared to the pre-value, respectively, in conscious cynomolgus monkeys. Therefore, the high dose was set at 50 mg/kg (as quinidine) as a dose at which marked QTc prolongation is considered to be observed if PK after oral administration to common marmosets is similar with that in cynomolgus monkeys. To investigate dose-relationship, 10 and 2 mg/kg (as quinidine) were set as the middle and the low dose, respectively, with a common ratio of 5.

Verapamil hydrochloride (verapamil): Ando *et al.* (2005a) reported that oral administration of verapamil at 15 mg/kg did not affect QTc and blood pressure although heart rate was increased in conscious cynomolgus monkeys. Therefore, the low dose was set at 15 mg/kg (as verapamil) as the no observed effect level (NOEL) for ECG parameters if PK after oral administration to common marmosets is similar with that in cynomolgus monkeys. To investigate dose-relationship, 50 and 150 mg/kg (as verapamil) were set as the middle and the high dose, respectively, with a common ratio of approximately 3. General condition and ECG parameters were carefully assessed before increasing dose level.

Terfenadine: Ando *et al.* (2005a) reported that oral administration of terfenadine at 100 mg/kg did not affect QTc and general condition in conscious cynomolgus monkeys, in addition, plasma exposure level did not increase dose-dependently from 30 mg/kg to 100 mg/kg. Therefore, the low dose was set at 30 mg/kg as the NOEL for ECG parameters even in case that plasma exposure level increases dose-dependently in common marmoset at over 30 mg/kg. To investigate dose-relationship, 100 and 300

mg/kg were set as the middle and the high dose, respectively, with a common ratio of approximately 3.

Clinical observation

On the dosing days, cage-side observations were conducted in telemetered common marmosets at pre-dose and after the completion of each telemetry data recording. General condition of telemetered common marmosets was observed via monitor through telemetry data recording. General condition of telemetered common marmosets was further confirmed by using recorded video images. On the other days, cage-side observations were conducted once daily. As for the common marmosets, which were used for TK examination, cage-side observations were conducted at pre-dose and at collecting blood samples on the dosing day. On the other days, cage-side observations were conducted once daily for each common marmoset.

Blood collection for determination of plasma concentrations

Three male animals, not among those used in the telemetry measurement, were administered the same doses of test article suspensions in a dose-escalation manner. Blood was collected from femoral vein for approximately 0.3 mL at each TK time point by using disposal heparinized syringes and needles (25 or 26 G), and the plasma was obtained after centrifugation at 4°C at $1,600 \times g$ for 5 min. All plasma samples were stored frozen until TK analysis.

TK analysis

Plasma concentrations of sotalol, astemizole, desmethyldastemizole, flecainide, quinidine, verapamil and terfenadine were determined using LC-MS/MS methods (see Analytical procedure). A 20–50 μ L of plasma and 200 μ L of internal standard solution (1 μ M phenytoin in methanol) were mixed well and kept at -20°C for 30 min and then centrifuged at $1,800 \times g$ for 10 min to remove precipitated protein. The supernatants were diluted 3-fold with distilled water for LC-MS/MS. Maximum plasma concentration (C_{max}) and the time to reach C_{max} after dosing (T_{max}) were calculated for each test article-treated group using the individual quantitative values obtained at each time point. Unbound C_{max} was calculated using the following formula:

$$\text{unbound } C_{\text{max}} = \text{total } C_{\text{max}} \times (100 - \text{serum protein binding ratio}) / 100$$

Serum protein binding ratio

In vitro protein binding of each test article using mar-

mosets serum was determined using the high-throughput dialysis method. Dialysis membranes had a 10-kDa molecular mass cutoff and were purchased from Harvard Apparatus Inc. (Harvard Apparatus Inc., Massachusetts, MA, USA). Marmoset serum containing the test articles (3.3–10 μ M, final concentration) was added to the acceptor chambers and phosphate-buffered saline (PBS) buffer (pH=7.4) was added to the donor chambers. The dialysis plate was placed in an incubator at 37°C for 22 hr on a plate rotator. After equilibrium had been reached, 10 μ L of samples in the acceptor chamber were mixed with 40 μ L of PBS buffer and 40 μ L of samples in the donor chamber were mixed with 10 μ L of marmoset serum. These samples were then mixed with 300 μ L of internal standard solution (1 μ M phenytoin or 1 μ M sulfaphenazole in methanol), and centrifuged at $1,800 \times g$ for 10 min to remove precipitated protein. The supernatants were diluted 3-fold with distilled water for LC-MS/MS (see Analytical procedure). Protein binding ratio was calculated using the following formula:

$$\text{protein binding ratio} = [1 - A_{\text{donor}} / (4 \times A_{\text{acceptor}})] \times 100$$

where A_{donor} and A_{acceptor} represent the peak area ratios of compounds to internal standard on donor and acceptor samples.

Analytical procedure

Concentrations of compounds in samples were measured by the LC-MS/MS method consisting of an API4000, API3200Qtrap or API5500Qtrap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with the Shimadzu 10A or 20A series HPLC system (Shimadzu, Kyoto, Japan). The analytical conditions of HPLC were shown in Supplemental Table 1. Mass spectrometric detection was conducted by positive or negative ionization electrospray. The selective reaction monitoring mode was used as follows to monitor ions (m/z : precursor ion / product ion): sotalol (273.2 / 133.4), astemizole (459.4 / 135.2), desmethyldastemizole (445.2 / 204.2), flecainide (415.1 / 301.2), quinidine (325.4 / 79.1), verapamil (455.4 / 165.3), terfenadine (472.4 / 436.2), phenytoin (253.2 / 182.2) and sulfaphenazole (315.0 / 158.0).

Test articles

Astemizole, flecainide acetate (flecainide), dl-sotalol hydrochloride (sotalol), terfenadine, and verapamil hydrochloride (verapamil) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Quinidine sulfate dihydrate (quinidine) was purchased from Wako Pure Chemical Industries (Osaka, Japan). O-desmethy-

lastemizole (desmethylastemizole) was purchased from Toronto Research Chemicals (North York, ON, Canada). Methylcellulose was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan).

Statistical analysis

As for PR interval, QRS duration and QTc in aggregated vehicle control data, statistical comparison between each time points (absolute values and change values from the pre-value) was conducted with one-way analysis of variance followed by Tukey's procedure. Statistical comparison of PR interval, QRS duration and QTc (absolute values and change values from the pre-value) between the vehicle control and each test article-treated group was conducted using a Dunnett-type multiple comparison test. The differences were evaluated at the two-tailed 5% level of statistical significance.

Regarding the ECG parameters, which were significantly changed by test article dosing, linear regression equations for the ECG parameter change against the plasma exposure level or body temperature were obtained by the least-squares method. The equation and R^2 was expressed.

ECG data are expressed as the mean $\pm$ standard deviation (S.D.). All statistics were calculated by using SAS software (version 9.2, SAS Institute, Cary, NC, USA).

RESULTS

ECG waveforms, recorded in this study

Typical ECG wave forms in common marmosets were demonstrated in Fig. 1. The waveforms recorded in this study were similar to those with lead II in healthy humans. PR interval, QRS duration, QT interval and RR interval were determined as shown in Fig. 1.

Circadian changes and individual differences in ECG parameters in the vehicle-treated conscious common marmosets

A total of 18 vehicle control ECG parameters were aggregated using the absolute values and changes from each pre-dose value (Δ value) at each time point (Table 2). No statistically significant change was observed in PR interval, QRS duration and QTc in comparison between each time points for both the absolute values and the Δ values. The S.D. values of the absolute values of PR interval, QRS duration and QTc were approximately 2-fold larger than those of the Δ values.

Effects of test articles on ECG parameters

Sotalol (Fig. 2, Table 3, Supplemental Table 2)

In the evaluation using the absolute values, no significant change in any ECG parameter was noted. The evaluation using the Δ values revealed PR prolongation at 2 hr post-dose at 5 mg/kg, and QTc prolongation at 1 hr post-dose at 5 mg/kg and at 1, 2, 4 and 8 hr post-dose at 15 mg/kg. No change in QRS duration was noted at any dose. Changes were not observed in general health condition and behavior during experiments at any doses. The C_{max} was 4,088 and 6,633 ng/mL at 5 and 15 mg/kg, respectively, and the T_{max} was 2 and 4 hr at 5 and 15 mg/kg, respectively. These findings indicate that sotalol induced dose-dependent QTc prolongation in conscious common marmosets, although no effect was observed on PR interval and QRS duration up to 15 mg/kg.

Astemizole (Fig. 2, Table 3, Supplemental Table 3)

Evaluation using the absolute values revealed QTc prolongation at 2, 4 and 6 hr post-dose at 30 mg/kg. No significant change in PR interval or QRS duration was noted at any dose. In the evaluation using the Δ values, QTc prolongation was noted at 1, 2, 4, 6 and 24 hr post-dose at 30 mg/kg. No significant change in PR interval or QRS duration was noted at any dose. Changes were not observed in general health condition and behavior during experiments at any doses. As for astemizole, the C_{max} was 0.3, 5.8 and 31 ng/mL at 3, 10 and 30 mg/kg, respectively, and the T_{max} was 3, 1 and 2.7 hr at 3, 10 and 30 mg/kg, respectively. As for desmethylastemizole, the

Fig. 1. Typical ECG waveforms in common marmosets. Typical tracing of the lead II electrocardiogram (ECG). PR, RR and QT intervals and QRS width are defined by ambilateral arrows.

Table 2. Aggregated ECG parameters of the vehicle control in absolute value (A) and change from pre-value (B) in common marmosets.

(A) Absolute value									(B) Change from pre value								
PR (msec)									PR (msec)								
Time post-dosing (hr)									Time post-dosing (hr)								
Time	Pre	1	2	4	6	8	24	Total	Time	Pre	1	2	4	6	8	24	Total
N	18	18	18	18	15	3	18	108	n	0	18	18	18	15	3	18	90
Mean	50	50	50	50	51	52	51	50	Mean	-	0	0	0	1	1	0	0
S.D.	4	4	5	5	5	7	5	5	S.D.	-	2	2	2	2	1	2	2
Max	56	56	57	57	55	58	56	58	Max	-	5	3	3	6	2	6	6
Min	42	43	41	40	40	45	41	40	Min	-	-3	-3	-3	-3	0	-4	-4

QRS (msec)									QRS (msec)								
Time post-dosing (hr)									Time post-dosing (hr)								
Time	Pre	1	2	4	6	8	24	Total	Time	Pre	1	2	4	6	8	24	Total
N	18	18	18	18	15	3	18	108	n	0	18	18	18	15	3	18	90
Mean	27	26	27	26	27	24	27	27	Mean	-	0	0	0	0	0	0	0
S.D.	2	2	2	2	2	3	3	2	S.D.	-	1	1	1	1	1	1	1
Max	29	29	29	29	30	27	29	30	Max	-	2	2	1	1	1	2	2
Min	22	22	21	22	21	21	20	20	Min	-	-1	-1	-1	-1	-1	-2	-2

QTc (msec)									QTc (msec)								
Time post-dosing (hr)									Time post-dosing (hr)								
Time	Pre	1	2	4	6	8	24	Total	Time	Pre	1	2	4	6	8	24	Total
N	18	18	18	18	15	3	18	108	n	-	18	18	18	15	3	18	90
Mean	146	144	145	143	148	143	148	145	Mean	-	-2	-1	-3	1	0	2	-1
S.D.	11	11	13	12	13	10	14	12	S.D.	-	4	5	4	5	7	6	5
Max	172	163	171	165	180	150	188	188	Max	-	4	5	4	9	8	16	16
Min	123	115	115	117	132	131	133	115	Min	-	-10	-10	-10	-6	-6	-9	-10

N: Number of animals, Mean: Average value, S.D.: Standard deviation, Max: Maximum value, Min: Minimum value.

$C_{\max}$ was 3.4, 16 and 61 ng/mL at 3, 10 and 30 mg/kg, respectively, and the $T_{\max}$ was 1, 2.7 and 1 hr at 3, 10 and 30 mg/kg, respectively. These findings indicate that astemizole induced QTc prolongation dose-dependently in conscious common marmosets, and no effect on PR interval and QRS duration up to 30 mg/kg.

Flecainide (Fig. 2, Table 3, Supplemental Table 4)

Evaluation using the absolute values affirmed PR prolongation at 1 and 2 hr post-dose at 45 mg/kg. In addition, QRS widening and QTc prolongation were noted at 1, 2 and 4 hr post-dose at 45 mg/kg. In the evaluation using the Δ values, PR prolongation and QRS widening were observed at 1, 2 and 4 hr post-dose at 45 mg/kg. Moreover, QTc prolongation was noted at 4 hr post-dose at 15 mg/kg, and at 1, 2 and 4 hr post-dose at 45 mg/kg. Flecainide at 45 mg/kg caused vomiting within 1 or 2 hr post-dose in all animals (data not shown). The $C_{\max}$ was 84, 1,407 and 4,140 ng/mL at 5, 15 and 45 mg/kg, respectively, and $T_{\max}$ was 1, 1.7 and 1.3 hr at 5, 15 and 45 mg/kg,

respectively. These findings indicate that flecainide dose-dependently caused QTc prolongation, PR prolongation and QRS widening in conscious common marmosets.

Quinidine (Fig. 3, Table 3, Supplemental Table 5)

In the evaluation using the absolute values, QRS widening was noted at 2 and 4 hr post-dose at 50 mg/kg and QTc prolongation was noted at 1, 2, 4 and 6 hr post-dose at 50 mg/kg. No significant change in PR interval was noted at any dose. In the evaluation using the Δ values, QRS widening was observed at 2 hr post-dose at 50 mg/kg and QTc prolongation was noted at 2 hr post-dose at 10 mg/kg and at 1, 2, 4 and 6 hr post-dose at 50 mg/kg. No significant change in PR interval was noted at any dose. At 1 and 2 hr post-dose at 50 mg/kg, ECG parameters from only 2 animals were available, because the ECG parameters of the other animal could not be determined due to marked tachycardia (not TdP). Therefore, ECG data for 50 mg/kg at the indicated time points were not included in statistical analysis. In the TK group, vomiting

Assessment of ECG parameters in common marmosets

Fig. 2. Effects of sotalol, astemizole and flecainide on ECG parameters in common marmosets. Time courses of QTc, PR interval and QRS duration after oral administration of sotalol (A), astemizole (B) and flecainide (C) are shown in absolute value and in change from pre-values. Circle symbols represent the vehicle control. Triangle symbols represent the first dose. Square symbols represent the second dose. Diamond symbols represent the third dose. The dotted line represents the mean $\pm 2 \times$ S.D. of the aggregated vehicle control data. Closed symbols represent statistically significant changes from the vehicle control ($p < 0.05$, compared to data from the vehicle-treated group). All data are presented as mean.

Table 3. Plasma exposure levels at each time point after oral administration of test articles.**Plasma exposure level (ng/mL)**

Test article	Dose level	Time post-dosing				C _{max}	T _{max} (hr)
		1 hr	2 hr	4 hr	8 hr		
Sotalol	5 mg/kg	3,235	4,088	2,779	1,381	4,088	2.0
	15 mg/kg	4,435	6,619	6,596	4,029	6,633	4.0
<hr/>							
Test article	Dose level	Time post-dosing				C _{max}	T _{max} (hr)
		1 hr	2 hr	4 hr	6 hr		
Astemizole	3 mg/kg	0.28	0.39	N.A.	0.24	0.3	3.0
	10 mg/kg	5.8	2.8	1.6	1.3	5.8	1.0
	30 mg/kg	31	22	14	11	31	2.7
Desmethylastemizole	3 mg/kg	3.4	2.7	1.3	1.3	3.4	1.0
	10 mg/kg	15	11	6.7	8.8	16	2.7
	30 mg/kg	61	49	31	26	61	1.0
Flecainide	5 mg/kg	84	71	19	N.D.	84	1.0
	15 mg/kg	1,353	1,337	612	N.D.	1,407	1.7
	45 mg/kg	4,090	3,897	3,337	N.D.	4,140	1.3
Quinidine	2 mg/kg	476	520	315	178	520	1.7
	10 mg/kg	1,553	1,827	1,307	848	1,827	2.0
	50 mg/kg	2,700	2,473	2,170	1,717	2,700	1.0
Verapamil	15 mg/kg	176	71	25	11	176	1.0
	50 mg/kg	599	287	106	51	599	1.0
	150 mg/kg	1,567	999	568	474	1,567	1.0
Terfenadine	30 mg/kg	12	8.7	7.2	6.2	13	2.7
	100 mg/kg	34	45	36	29	48	2.7
	300 mg/kg	57	97	131	93	135	3.3

N.A.: Not applicable. N.D.: No data.

was observed within 1 hr post-dose in 1 out of 3 animals at 10 mg/kg, and in 2 out of 3 animals at 50 mg/kg (data not shown). The C_{max} was 520, 1,827 and 2,700 ng/mL at 2, 10 and 50 mg/kg, respectively, and the T_{max} was 1.7, 2 and 1 hr at 5, 25 and 125 mg/kg, respectively. These findings indicate that quinidine induced dose-dependent QTc prolongation and QRS widening in conscious common marmosets, however, no change was noted in PR interval up to 50 mg/kg.

Verapamil (Fig. 3, Table 3, Supplemental Table 6)

In the evaluation using the absolute values, QTc prolongation was noted at 2 and 4 hr post-dose at 150 mg/kg. No significant change in PR interval or QRS duration was observed at any dose. In the evaluation using the Δ values, PR prolongation was observed at 1, 2 and 4 hr post-dose at 150 mg/kg and QTc was prolonged at 2 and 4 hr post-dose at 150 mg/kg. No significant change in QRS duration was noted at any dose. Changes were not observed in general health condition and behavior during experiments at any doses. The C_{max} was 176, 599 and 1,567 ng/mL at 15, 50 and 150 mg/kg, respectively and the T_{max} was 1 hr

at all dose levels. These findings indicate that verapamil induced dose-dependent QTc prolongation and PR prolongation in conscious common marmosets, and no effect on QRS duration up to 150 mg/kg.

Terfenadine (Fig. 3, Table 3, Supplemental Table 7)

In the evaluation using the absolute values, QTc prolongation was noted at 4 hr post-dose at 100 mg/kg and at 1, 2, 4 and 6 hr post-dose at 300 mg/kg. No significant change in PR interval or QRS duration was noted at any dose. In the evaluation using the Δ values, QTc was prolonged at 4 hr post-dose at 30 and 100 mg/kg, and at 1, 2, 4 and 6 hr post-dose at 300 mg/kg. No change in PR interval or QRS duration was observed even at 300 mg/kg. QRS widening, which was noted at 4 hr post-dose at 30 mg/kg, was not considered test article-related, because it was not dose-dependent. Changes were not observed in general health condition and behavior during experiments at any doses. The C_{max} was 13, 48 and 135 ng/mL at 30, 100 and 300 mg/kg, respectively, and the T_{max} was 2.7, 2.7 and 3.3 hr at 30, 100 and 300 mg/kg, respectively. These findings indicate that terfenadine induced dose-de-

Assessment of ECG parameters in common marmosets

Table 4. Plasma unbound levels of test articles showing changes in ECG parameters in common marmosets, beagle dogs, cynomolgus monkeys, and humans.

Test article	Changed ECG parameter	Plasma levels showing the ECG changes (ng/mL)			Ratio		Reference
		CM	Human	Dog & Monkey	CM / Human	CM / Dog & Monkey	
Sotalol	QTc prolongation	3875	1,008-2,996	(D) 3,073	1.3-3.8	1.3	Human: Redfern <i>et al.</i> , 2003 Human: Gotta <i>et al.</i> , 2016 Dog: Himmel <i>et al.</i> , 2012
Astemizole	QTc prolongation	0.247	N.A.	N.A.	N.C.	N.C.	Human: Hoppu <i>et al.</i> , 1991 Dog: Toyoshima <i>et al.</i> , 2005 Monkey: Ando <i>et al.</i> , 2005a
Flecainide	QTc prolongation	625	660	N.D.	1	N.C.	Human: Salerno <i>et al.</i> , 1986
	PR prolongation	1,838	669	(D) 996	2.7	1.8	Human: Bergenholm <i>et al.</i> , 2017
	QRS widening	1,838	669	(D) 256	2.7	7.2	Dog: Himmel <i>et al.</i> , 2012
Quinidine	QTc prolongation	163	97-224	(D) 395 (M) 166	0.7-1.7	0.4-1.0	Human: Redfern <i>et al.</i> , 2003 Human: Bergenholm <i>et al.</i> , 2017 Dog: Toyoshima <i>et al.</i> , 2005
	QRS widening	240	599	(D) 908 (M) N.D.	0.4	0.3	Dog: Cros <i>et al.</i> , 2012 Monkey: Ando <i>et al.</i> , 2005a Monkey: Akabane <i>et al.</i> , 2010
Verapamil	QTc prolongation	45	N.D.	N.D.	N.C.	N.C.	Human: Bergenholm <i>et al.</i> , 2017
	PR prolongation	45	94	(D) 17.6 (M) N.D.	0.5	2.6	Dog: Himmel <i>et al.</i> , 2012 Monkey: Ando <i>et al.</i> , 2005a
Terfenadine	QTc prolongation	0.05	0.09	(D) 6.52 (M) N.A.	0.6	0.008	Human: Redfern <i>et al.</i> , 2003 Dog: Van Deuren <i>et al.</i> , 2009 Dog: Webster <i>et al.</i> , 2001 Monkey: Ando <i>et al.</i> , 2005b

Data for common marmosets are based on the results of this study. Data for beagle dog, cynomolgus monkeys and humans are reported in the literature. CM: Common marmoset. N.A.: Not available. N.D.: Not detected. N.C.: Not calculated. (D): Dog data. (M): Monkey data.

pendent QTc prolongation in conscious common marmosets, and no change was observed in PR interval and QRS duration up to 300 mg/kg.

Test articles plasma exposure levels at doses that induced ECG changes

The changes in ECG parameters noted in the present study and the unbound $C_{\max}$ value at the lowest observed effective dose level (LOEL) for each test article are shown in Table 4. For comparison purposes, the reported relationships between changes in ECG parameters and effective plasma levels for each test article in humans and dogs/monkeys are also described. The unbound $C_{\max}$ values in common marmosets were calculated using plasma protein binding ratio in common marmosets (Supplemental Table 8) and the plasma level data. All test articles (sotalol, astemizole, flecainide, quinidine, verapamil and terfenadine) caused QTc prolongation at unbound $C_{\max}$ values of 3,785, 0.247, 625, 163, 45, and 0.05 ng/mL, respectively, in common marmosets. Differences in these concentrations for sotalol, flecainide, quinidine and ter-

fenadine between common marmosets and human are 0.6 to 3.8-fold. Although verapamil caused QTc prolongation in the present study, this effect has not been reported in humans. As the astemizole-induced QTc prolongation in humans has been assessed by using the sum of plasma exposure levels of astemizole itself and its major metabolite, the unbound plasma exposure level of astemizole alone was not used in this evaluation. As for the other changes observed with flecainide (PR prolongation and QRS widening), quinidine (QRS widening), and verapamil (PR prolongation), the plasma levels at the effective doses in common marmosets were 0.4 to 2.7-fold of those in humans. In a comparison with dogs and monkeys, difference of plasma level at the ECG effective dose was 0.3 to 7.2-fold except for QTc prolongation by astemizole, flecainide, verapamil and terfenadine. Terfenadine-induced QTc prolongation was associated with much lower plasma level in common marmosets than in dogs (0.008-fold). Astemizole was not included in the above comparison, because its unbound plasma level at the effective dose in dogs and monkeys could not be determined from

Fig. 3. Effects of quinidine, verapamil and terfenadine on ECG parameters in common marmosets. Time courses of QTc, PR interval and QRS duration after oral administration of quinidine (A), verapamil (B) and terfenadine (C) are shown in absolute value and in change from pre values. Circle symbols represent the vehicle control. Triangle symbols represent the first dose. Square symbols represent the second dose. Diamond symbols represent the third dose. The dotted line represents the mean $\pm$ 2 $\times$ S.D. of the aggregated vehicle control data. Closed symbols represent statistically significant changes from the vehicle control ($p < 0.05$, compared to data from the vehicle-treated group). #: Judged to be significant. The symbols were filled since the changes noted in 2 common marmosets exceeded the range of any vehicle control data, although, statistical analysis was not conducted. All data are presented as mean.

previous reports. QTc prolongation by flecainide or verapamil could not be compared because those effects in dogs and monkeys have not been reported.

Relationship between decrease in body temperature and QTc change after verapamil treatment

Regarding to verapamil-induced QTc prolongation noted in common marmosets, a body temperature dependency was investigated. Fig. 4 shows the relationships between changes in body temperature and QTc after verapamil treatment. It was revealed that QTc prolongations after verapamil treatment were observed under body temperature decreased condition.

Relationship between test articles plasma exposure level and ECG parameters changes

Changes in ECG parameters in the absolute values or the Δ values and the corresponding unbound plasma concentrations at each TK time point are plotted in Fig. 5. The ECG parameters changes showed positive correlation to the unbound plasma exposure level, except for PR interval change by sotalol, and their R^2 was 0.3722-0.9273 in the absolute value analysis and 0.5640-0.9365 in the Δ value analysis. As regard to the change in PR interval by sotalol, the slope was negative although statistically significant prolongation was detected at low dose level.

Regarding QTc prolongation, 88% (23/26) of the statistically significant points fell above the dotted line indicating the mean + 2 $\times$ S.D. of the vehicle control data in the evaluation using the Δ values and 71% (12/17) of

those fell above the dotted line in the evaluation using the absolute values. Regarding PR prolongation and QRS widening, almost all changes that were larger than the mean + 2 $\times$ S.D. of the aggregated vehicle control data were significant in both analysis using the absolute values and the Δ values.

DISCUSSION

Circadian changes and variations in ECG parameters in telemetered common marmosets

To better characterize the common marmoset telemetry model, we first confirmed the effects of circadian changes on PR interval, QRS duration and QTc using the aggregated vehicle control data. We found no obvious circadian-related changes in PR interval, QRS duration or QTc, in the light period and these results were similar to previous reports (Horii *et al.*, 2002; Tabo *et al.*, 2008). Therefore, circadian changes are not considered to influence ECG parameters in common marmosets, in case evaluation time points are set appropriately in the light period.

Usefulness of criteria based on historical vehicle control data for judgment of test article related change in ECG parameters

Less variability of ECG parameters was found in the Δ values at all the time points, when compared the S.D. values of the Δ value and the absolute value in the aggregated vehicle control data. Because the use of small valuable parameter is possible to detect small changes, we selected the use of the Δ values in addition to that of absolute values in our assessment. Actually, the ECG changes were detected at lower dose and at more time points in the Δ value evaluation in the present study (Table 5). Therefore, it is considered that the use of the Δ values can detect ECG parameter changes with higher sensitivity than the use of absolute values.

As shown in Fig. 5, almost all points showing significant change in QTc, PR interval and QRS duration, are plotted above the mean + 2 $\times$ S.D. of the vehicle control data. Especially in the Δ value analysis, all the significant changes, except for 3 points in QTc, were larger than the mean + 2 $\times$ S.D. Therefore, it is suggested that the mean + 2 $\times$ S.D. of the vehicle control data can be useful as a criterion of ECG parameters changes, although it should be noted that the statistical power in the present study design ($n = 3$, using ascending dose manner) is considered lower than that in the golden standard telemetry study design ($n = 4$, using 4 $\times$ 4 Latin square dosing manner).

Fig. 4. Relationship between QTc and body temperature after oral administration of verapamil in common marmosets. The relationship between QTc and body temperature after oral administration of verapamil is shown. Individual common marmoset data at all the evaluation time points are plotted.

Fig. 5. Relationship between change in ECG parameters and test articles plasma exposure levels in common marmosets. The relationships between test articles plasma exposure levels and changes in QTc, PR interval, and QRS duration are shown in the absolute values or the change from pre-value. Closed symbols represent statistically significant changes from the vehicle control ($p < 0.05$, compared to data from the vehicle-treated group). #: Judged as significant since the changes noted in 2 common marmosets exceeded the range of any vehicle control data, although statistical analysis was not conducted.

PR prolongation

Flecainide and verapamil were reported to prolong PR interval in humans, but the prolongation was not evident in clinical use of the other compounds used in this study (Table 4). Similar to humans, common marmosets showed

dose-dependent PR prolongation by flecainide or verapamil administration, but not by the other compounds. These findings indicate that PR prolongations induced by multiple cardiac ion channel inhibitors were detected in conscious common marmosets similar to humans.

Table 5. Comparison of LOEL and number of effective time points for ECG changes between the evaluations using the absolute values and the Δ values.

Test article	ECG parameter	LOEL (mg/kg)		Number of time points at which ECG change was noted	
		A	Δ	A	Δ
Sotalol	QTc	-	15	0	5
Astemizole	QTc	30	30	3	4
Flecainide	QTc	45	15	3	4
	PR	45	45	2	3
	QRS	45	45	3	3
Quinidine	QTc	50	10	4	5
	QRS	50	50	2	1
Verapamil	QTc	150	150	2	2
	PR	-	150	0	3
Terfenadine	QTc	100	30	5	6

LOEL: the lowest observed effect level. A: Absolute value. Δ : Change from pre value.

Flecainide and verapamil

Both flecainide and verapamil exposure-dependently prolonged PR interval in common marmosets, and these effects were also reported in humans (Table 4). The unbound plasma exposure level of flecainide and verapamil at PR prolonged dose in the common marmoset and humans were similar (differences were 0.5 to 2.7-fold). These effects were also reported in dogs at similar unbound plasma levels (Himmel *et al.*, 2012). Therefore, PR prolongation induced by the test articles is considered well detected in common marmosets at plasma exposure levels similar with those in humans and dogs.

PR prolongation has been reported to be related to Nav1.5 and Cav1.2 inhibitions (Priest and McDermott, 2015; Bergenholm *et al.*, 2017). Plasma exposure-dependent PR prolongation by verapamil is considered due to Cav1.2 inhibition because verapamil has strong inhibitory potentials to hERG and Cav1.2 and the other hERG positive compounds except for flecainide did not induce PR prolongation at QTc prolonged dose. In addition, the relationship between PR prolongation and estimated Cav1.2 inhibition in humans was demonstrated using verapamil (Bergenholm *et al.*, 2017). The flecainide-induced PR prolongation is possible to be related to dual effects of Nav1.5 and Cav1.2 inhibitions because flecainide has both Nav1.5 and Cav1.2 inhibitory potentials. It is suggested that the contribution of the effect of Nav1.5 inhibition on PR prolongation can be estimated by assessing the effect on P wave duration in guinea pig heart (Isobe *et al.*, 2018), however, the effects on P wave duration was not assessed in the present common marmoset study, indicating that the contribution of Nav1.5 or Cav1.2 inhibitions to flecainide-induced PR prolongation in common marmoset is unclear. It is suggested that PR prolongation

observed in the present study is caused by well-known mechanism that is Nav1.5 and/or Cav1.2 inhibitions.

Sotalol

Only one point at the low dose was statistically significant in the Δ value analysis (Fig. 2). The PR prolongation, however, seemed not to be related to the pharmacological action of sotalol, because it was not detected at the high dose and no plasma exposure relationship was found in the PR changes (Fig. 5). This finding might be also supported by previous reports that sotalol did not affect PR interval up to the plasma exposure level of 6 $\mu\text{g/mL}$ in humans (McComb *et al.*, 1987; Sahar *et al.*, 1989).

The other test article (astemizole, quinidine and terfenadine)

The other test articles did not induce PR prolongation in common marmosets and these compounds have not been well reported to induce PR prolongation in humans. Therefore, it is suggested that the results of astemizole, quinidine and terfenadine in this study are similar to those in the previous investigation in clinic.

QRS widening

Flecainide and quinidine were reported to induce QRS widening in humans, but there is no evidence of the widening in clinical use of the other compounds used in this study (Table 4). Similar to humans, common marmosets showed dose-dependent QRS widening by flecainide or quinidine administration, but not in the other compounds. The plasma exposure levels of flecainide and quinidine was over one twentieth of their 50% inhibitory concentration (IC_{50}) of Nav1.5, whereas those of the other compounds were lower than 1/300 of their Nav1.5 IC_{50} in this

study. These findings indicate that QRS widening, which might be induced by Nav1.5 inhibition, were detected in conscious common marmosets.

Flecainide and quinidine

Both flecainide and quinidine exposure-dependently induced QRS widening in common marmosets (Fig. 5), and these effects were also reported in humans (differences were 0.4 to 2.7-fold, Table 4). Although statistical comparison was not conducted at 2 hr post-dose of quinidine at 50 mg/kg due to the occurrence of marked tachycardia in one marmoset, the QRS widening at the point was considered significant, because data from the other 2 marmosets revealed marked QRS widening that exceeded the range of any vehicle control data. The unbound plasma level of flecainide and quinidine at the effective dose in common marmosets were similar to those in humans (differences were 0.4 to 2.7-fold). The QRS widening was also reported in dogs at similar plasma levels (Himmel *et al.*, 2012; Toyoshima *et al.*, 2005; Cros *et al.*, 2012). Therefore, QRS widening induced by the test articles is considered well detected in common marmosets at plasma exposure levels similar with that in humans and dogs.

Flecainide- and quinidine-induced QRS widening is suggested to be related to Nav1.5 channel inhibition, because the compounds have relatively strong Nav1.5 inhibitory potential (known as Class I arrhythmic drugs) and the relationship between QRS widening and estimated Nav1.5 inhibition in humans was demonstrated using flecainide and quinidine (Bergenholtz *et al.*, 2017). The plasma exposure levels of flecainide or quinidine reached at 4.4 μM or 0.7 μM , respectively, which were similar to and one twentieth at their IC_{50} for Nav1.5, respectively. On the other hand, the other test articles have much weaker Nav1.5 inhibitory potential compared to hERG inhibitory potential and did not cause QRS widening. The plasma exposure levels of these compound were below 1/300 of their Nav1.5 IC_{50} in this study, and the levels are much lower than those of flecainide and quinidine. Therefore, QRS widening observed in the present study is suggested to be caused by well-known mechanism of Nav1.5 inhibition.

The other test article (sotalol, astemizole, verapamil and terfenadine)

The other test articles did not induce QRS widening in common marmosets, and these are considered in agreement with the previous reports demonstrating clinical ECG effects of these compounds. The plasma exposure levels of these compounds were lower than 1/300 of their

Nav1.5 IC_{50} even at the high dose in this study. Therefore, Nav1.5 inhibition by these compounds was considered not to be sufficient to induce QRS prolongation under the condition of this study.

QTc prolongation

All test articles were reported to have inhibitory potential for hERG, Nav1.5 and Cav1.2 (Supplemental Table 9). They induced QTc prolongation in common marmosets, and the compounds except for verapamil were reported to induce QTc prolongation in humans (Table 4). These finding indicates that QTc prolongations induced by hERG positive compounds are considered to be detected in conscious common marmosets, even if the compounds have other ion channels inhibitory potential. However, it should be noted that body temperature-related QTc prolongation can be easily observed in common marmosets because of their small body size.

Sotalol, flecainide and quinidine

Sotalol, flecainide and quinidine exposure-dependently prolonged QTc in this study (Fig. 5) and the plasma exposure level at the effective dose was similar to those in humans (differences were 0.7 to 3.8-fold, Table 4). Sotalol and quinidine-related QTc prolongation were detected in beagle dogs and cynomolgus monkeys at similar exposure levels (Himmel *et al.*, 2012; Toyoshima *et al.*, 2005; Ando *et al.*, 2005a). On the other hand, Himmel *et al.* (2012) reported that no QTc prolongation was noted in beagle dog up to 2,700 ng/mL although marked QRS widening was observed. Therefore, it is indicated that the QTc prolongations induced by sotalol, flecainide and quinidine are well detected in common marmosets at plasma exposure levels similar with that in humans.

Astemizole

Astemizole dose-dependently prolonged QTc. Plasma exposure level of astemizole and its major active metabolite, desmethylastemizole, were measured in our study, because both of them have reported to inhibit hERG channel with similar IC_{50} values (Zhou *et al.*, 1999). As the proarrhythmic potential of astemizole in humans has been assessed by the sum of plasma exposure levels of astemizole and desmethylastemizole (Hopppu *et al.*, 1991; Saviuc *et al.*, 1993), the sum was also used for the comparisons of the QTc and the exposure level in this study (Table 6). Although the ratio of astemizole and desmethylastemizole in plasma concentration has been reported to be different among animal species, degrees of their QTc prolongation were similar between humans, dogs, monkeys

Table 6. Plasma concentration of astemizole and desmethylastemizole at QTc prolonged dose in humans, common marmosets, monkeys and dogs.

Species	Unbound plasma concentration (ng/mL)		Total plasma concentration (ng/mL)			Reference
	A	D	A	D	A + D	
Common marmoset	0.247	1.15	31	61	92	Results of the present study
Human	N.A.	N.A.	N.A.	N.A.	15.9-250	Hoppu <i>et al.</i> , 1991 Saviuc <i>et al.</i> , 1993
Common marmoset	0.138	4.00	11.5	119	131	Tabo <i>et al.</i> , 2008
Cynomolgus monkey	N.A.	N.A.	10	230	240	Ando <i>et al.</i> , 2005a
Beagle dog	N.A.	N.A.	13	15	28	Toyoshima <i>et al.</i> , 2005

LVH: left ventricular hypertrophy, A: astemizole, D: desmethylastemizole, Ref: Reference N.A.: Not available

and common marmosets (Toyoshima *et al.*, 2005; Ando *et al.*, 2005a; Tabo *et al.*, 2008). Therefore, to use their sum of exposure level is suitable to compare the QTc prolongation by astemizole in different species.

Verapamil

QTc prolongation was detected in the present study after oral administration of verapamil, although this change has not been reported in humans, dogs or monkeys (Table 4). The QTc prolongation after verapamil administration in common marmosets was associated with decrease in body temperature (Fig. 4), and it has been reported that 2 or 3 degrees decrease in body temperature can induce QTc prolongation in dogs and humans (van der Linde *et al.*, 2008; El Amrani *et al.*, 2016; Lions *et al.*, 2018). In addition, small body size animals, which indicate high ratio of body surface area to body weight, are considered to more easily lose their bodily heat than large body size ones (Hegarty *et al.*, 2009; Fosgerau *et al.*, 2010). The unbound plasma exposure level at QTc prolongation dose in common marmosets (45 ng/mL = 99 nM) reached 1/3 of IC₅₀ for hERG channel inhibition (250 nM) (Kramer *et al.*, 2013) in the present study. Therefore, the QTc prolongation at 150 mg/kg of verapamil might be related to the decrease in body temperature, leading to be a cause of the species difference in the prolongation. In addition, hERG inhibition is also possible to be related to the prolongation because verapamil has a strong inhibitory potential to hERG channel.

Terfenadine

Terfenadine dose-dependently prolonged QTc in common marmosets and the plasma exposure level at the effective dose was similar to those in humans (differences were 0.6-fold, Table 4). In comparison with the other animal species, a large difference in sensitivity was suggested between common marmosets and dogs (differences were 0.008-fold, Table 4). The low sensitivity to terfenadine-induced QTc prolongation in dogs has been suggested by previous studies, for example, QTc prolongation was observed at total plasma concentration of 326 ng/mL (unbound plasma level is calculated to 6.52 ng/mL), and was not observed at total plasma concentration of 181 ng/mL or less (Toyoshima *et al.*, 2005; Van Deuren *et al.*, 2009; Webster *et al.*, 2001). In monkeys, QTc prolongation was not noted after single oral administration of terfenadine due to low plasma exposure, which is related to a fast metabolic rate in cynomolgus monkeys (Ando *et al.*, 2005a), however, co-administration of ketoconazole, a strong CYP3A4 inhibitor, elevated total C_{max} of terfenadine to 26 ng/mL, leading to QTc prolongation (Ando *et al.*, 2005b). Total plasma level of terfenadine at QTc prolonged doses in common marmosets and monkeys were similar (45 and 26 ng/mL, respectively). Therefore, the common marmoset is considered a better experimental animal for the assessment of terfenadine-induced QTc prolongation than dogs and monkeys. Terfenadine is known to be converted to fexofenadine, an active metabolite, after oral dosing. However, only the plasma terfenadine level was used for the evaluation in this study because fexofenadine is indicated to have little effect on ECG (Ando *et al.*, 2018).

dine-induced QTc prolongation in dogs has been suggested by previous studies, for example, QTc prolongation was observed at total plasma concentration of 326 ng/mL (unbound plasma level is calculated to 6.52 ng/mL), and was not observed at total plasma concentration of 181 ng/mL or less (Toyoshima *et al.*, 2005; Van Deuren *et al.*, 2009; Webster *et al.*, 2001). In monkeys, QTc prolongation was not noted after single oral administration of terfenadine due to low plasma exposure, which is related to a fast metabolic rate in cynomolgus monkeys (Ando *et al.*, 2005a), however, co-administration of ketoconazole, a strong CYP3A4 inhibitor, elevated total C_{max} of terfenadine to 26 ng/mL, leading to QTc prolongation (Ando *et al.*, 2005b). Total plasma level of terfenadine at QTc prolonged doses in common marmosets and monkeys were similar (45 and 26 ng/mL, respectively). Therefore, the common marmoset is considered a better experimental animal for the assessment of terfenadine-induced QTc prolongation than dogs and monkeys. Terfenadine is known to be converted to fexofenadine, an active metabolite, after oral dosing. However, only the plasma terfenadine level was used for the evaluation in this study because fexofenadine is indicated to have little effect on ECG (Ando *et al.*, 2018).

Limitation

In this study, the ECG effects of 6 multiple cardiac ion channel inhibitors and the exposure relationship with the ECG changes were investigated. Some factors are known to have an influence on the compound's effects on ECG parameters such as animal sex, autonomic nervous activity, potential and metabolism of test articles, but these were not investigated in this study. The limited number of experimental animals could be related to low statistical power.

Conclusion

In the present study, we investigated the ECG effects

of 6 multiple cardiac ion channel inhibitors, namely sotalol, astemizole, flecainide, quinidine, verapamil and terfenadine, in conscious telemetered common marmosets, and the relationship between plasma exposure level and ECG parameters. Almost all the ECG changes induced by the compounds, including PR prolongation, QRS widening and QTc prolongation, were detectable in conscious common marmosets at exposure levels similar to those in humans, although QTc prolongation, which might be related to decrease in body temperature, can occur easily by reason of common marmosets' small body size. Therefore, the common marmoset telemetry study may be useful to assess the effects of multiple cardiac ion channel inhibitors on ECG parameters.

ACKNOWLEDGMENT

We thank Satoki Imai and Shun Hayashi for their great help with measurement of protein binding ratio and plasma concentration of test articles.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

- Akabane, T., Tabata, K., Kadono, K., Sakuda, S., Terashita, S. and Teramura, T. (2010): A comparison of pharmacokinetics between humans and monkeys. *Drug Metab. Dispos.*, **38**, 308-316.
- Ando, K., Hombo, T., Kanno, A., Ikeda, H., Imaizumi, M., Shimizu, N., Sakamoto, K., Kitani, S., Yamamoto, Y., Hizume, S., Nakai, K., Kitayama, T. and Yamamoto, K. (2005a): QT PRODACT: *in vivo* QT assay with a conscious monkey for assessment of the potential for drug-induced QT interval prolongation. *J. Pharmacol. Sci.*, **99**, 487-500.
- Ando, K., Unakami, S., Obo, M., Sasaki, A., Makita, Y., Yamamoto, Y. and Hayashi, K. (2005b): QT-prolongation effect of orally administered terfenadine in conscious cynomolgus monkeys –interaction with ketoconazole-. *Adv. Anim. Cardiol.*, **38**, 9-20.
- Ando, K., Nakamura, Y., Hagiwara-Nagasawa, M., Harada, H., Miyamoto, H., Inamura, N., Takagi, K., Goto, A., Chiba, K., Lubna, N.J., Izumi-Nakaseko, H., Naito, A.T. and Sugiyama, A. (2018): Comparison of electropharmacological effects between terfenadine and its active derivative fexofenadine using a crossover study in halothane-anesthetized dogs to analyze variability of pharmacodynamic and pharmacokinetic profiles of terfenadine and torsadogenic risk of fexofenadine. *J. Toxicol. Sci.*, **43**, 183-192.
- Bergenholt, L., Parkinson, J., Mettetal, J., Evans, N.D., Chappell, M.J. and Collins, T. (2017): Predicting QRS and PR interval prolongations in humans using nonclinical data. *Br. J. Pharmacol.*, **174**, 3268-3283.
- Cros, C., Skinner, M., Moors, J., Laine, P. and Valentin, J.P. (2012): Detecting drug-induced prolongation of the QRS complex: new insights for cardiac safety assessment. *Toxicol. Appl. Pharmacol.*, **265**, 200-208.
- El Amrani, A.I., El Amrani-Callens, F., Lorient, S., Singh, P. and Forster, R. (2016): QT interval correction for drug-induced changes in body temperature during integrated cardiovascular safety assessment in regulatory toxicology studies in dogs: A case study. *J. Pharmacol. Toxicol. Methods*, **81**, 136-143.
- Fosgerau, K., Weber, U.J., Gotfredsen, J.W., Jayatissa, M., Buus, C., Kristensen, N.B., Vestergaard, M., Teschendorf, P., Schneider, A., Hansen, P., Raunso, J., Køber, L., Torp-Pedersen, C. and Videbaek, C. (2010): Drug-induced mild therapeutic hypothermia obtained by administration of a transient receptor potential vanilloid type 1 agonist. *BMC Cardiovasc. Disord.*, **10**, 51.
- Gotta, V., Yu, Z., Cools, F., van Ammel, K., Gallacher, D.J., Visser, S.A., Sannajust, F., Morissette, P., Danhof, M. and van der Graaf, P.H. (2016): Application of a systems pharmacology model for translational prediction of hERG-mediated QTc prolongation. *Pharmacol. Res. Perspect.*, **4**, e00270.
- Heath, B.M., Cui, Y., Worton, S., Lawton, B., Ward, G., Ballini, E., Doe, C.P., Ellis, C., Patel, B.A. and McMahon, N.C. (2011): Translation of flecainide- and mexiletine-induced cardiac sodium channel inhibition and ventricular conduction slowing from nonclinical models to clinical. *J. Pharmacol. Toxicol. Methods*, **63**, 258-268.
- Hegarty, J., Walsh, E., Burton, A., Murphy, S., O'gorman, F. and McPolin, G. (2009): Nurses' knowledge of inadvertent hypothermia. *AORN J.*, **89**, 701-704, 707-713.
- Himmel, H.M., Bussek, A., Hoffmann, M., Beckmann, R., Lohmann, H., Schmidt, M. and Wettwer, E. (2012): Field and action potential recordings in heart slices: correlation with established *in vitro* and *in vivo* models. *Br. J. Pharmacol.*, **166**, 276-296.
- Holzgreffe, H.H., Caverio, I., Gleason, C.R., Warner, W.A., Buchanan, L.V., Gill, M.W., Burkett, D.E. and Durham, S.K. (2007): Novel probabilistic method for precisely correcting the QT interval for heart rate in telemetered dogs and cynomolgus monkeys. *J. Pharmacol. Toxicol. Methods*, **55**, 159-175.
- Honda, M., Komatsu, R., Holzgreffe, H.H., Yamada, Y., Isobe, T., Kimura, K., Itoh, T., Tamaoki, N. and Tabo, M. (2010): Application of probabilistic analysis for precisely correcting the QT interval for heart rate in telemetered common marmosets. *J. Pharmacol. Toxicol. Methods*, **61**, 264-270.
- Hoppu, K., Tikanoja, T., Tapanainen, P., Remes, M., Saarenpää-Heikkilä, O. and Kouvalainen, K. (1991): Accidental astemizole overdose in young children. *Lancet*, **338**, 538-540.
- Horii, I., Kito, G., Hamada, T., Jikuzono, T., Kobayashi, K. and Hashimoto, K. (2002): Development of telemetry system in the common marmoset--cardiovascular effects of astemizole and nicardipine. *J. Toxicol. Sci.*, **27**, 123-130.
- Isobe, T., Honda, M., Komatsu, R. and Tabo, M. (2018): Conduction and contraction properties of human iPS cell-derived cardiomyocytes: analysis by motion field imaging compared with the guinea-pig isolated heart model. *J. Toxicol. Sci.*, **43**, 493-506.
- Johnston, J., Pal, S. and Nagele, P. (2013): Perioperative torsade de pointes: a systematic review of published case reports. *Anesth. Analg.*, **117**, 559-564.
- Komatsu, R., Honda, M., Holzgreffe, H.H., Kubo, J., Yamada, Y., Isobe, T., Kimura, K., Itoh, T., Tamaoki, N. and Tabo, M. (2010): Sensitivity of common marmosets to detect drug-induced QT interval prolongation: moxifloxacin case study. *J. Pharmacol. Toxicol. Methods*, **61**, 271-276.
- Kramer, J., Obejero-Paz, C.A., Myatt, G., Kuryshev, Y.A., Bruening-Wright, A., Verducci, J.S. and Brown, A.M. (2013):

Assessment of ECG parameters in common marmosets

- MICE models: superior to the HERG model in predicting Torsade de Pointes. *Sci. Rep.*, **3**, 2100.
- Li, M. and Ramos, L.G. (2017): Drug-induced QT prolongation and torsades de pointes. *P&T*, **42**, 473-477.
- Li, Z., Ridder, B.J., Han, X., Wu, W.W., Sheng, J., Tran, P.N., Wu, M., Randolph, A., Johnstone, R.H., Mirams, G.R., Kuryshv, Y., Kramer, J., Wu, C., Crumb, W.J. Jr. and Strauss, D.G. (2019): Assessment of an in silico mechanistic model for proarrhythmia risk prediction under the CiPA initiative. *Clin. Pharmacol. Ther.*, **105**, 466-475.
- Lions, S., Dragu, R., Carsenty, Y., Zukermann, R. and Aronson, D. (2018): Determinants of cardiac repolarization and risk for ventricular arrhythmias during mild therapeutic hypothermia. *J. Crit. Care*, **46**, 151-156.
- McComb, J.M., McGovern, B., McGowan, J.B., Ruskin, J.N. and Garan, H. (1987): Electrophysiologic effects of d-sotalol in humans. *J. Am. Coll. Cardiol.*, **10**, 211-217.
- O'Hara, T., Virág, L., Varró, A. and Rudy, Y. (2011): Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS Comput. Biol.*, **7**, e1002061.
- Priest, B.T. and McDermott, J.S. (2015): Cardiac ion channels. *Channels (Austin)*, **9**, 352-359.
- Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., MacKenzie, I., Palethorpe, S., Siegl, P.K., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J. and Hammond, T.G. (2003): Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.*, **58**, 32-45.
- Sahar, D.I., Reiffel, J.A., Bigger, J.T. Jr., Squatrito, A. and Kidwell, G.A. (1989): Efficacy, safety, and tolerance of d-sotalol in patients with refractory supraventricular tachyarrhythmias. *Am. Heart J.*, **117**, 562-568.
- Saito, A. (2015): The marmoset as a model for the study of primate parental behavior. *Neurosci. Res.*, **93**, 99-109.
- Salerno, D.M., Granrud, G., Sharkey, P., Krejci, J., Larson, T., Erlien, D., Berry, D. and Hodges, M. (1986): Pharmacodynamics and side effects of flecainide acetate. *Clin. Pharmacol. Ther.*, **40**, 101-107.
- Saviuc, P., Danel, V. and Dixmerias, F. (1993): Prolonged QT interval and torsade de pointes following astemizole overdose. *J. Toxicol. Clin. Toxicol.*, **31**, 121-125.
- Tabo, M., Hara, T., Sone, S., Shishido, N., Kuramoto, S., Nakano, K., Onodera, H., Kimura, K. and Kobayashi, K. (2008): Prediction of drug-induced QT interval prolongation in telemetered common marmosets. *J. Toxicol. Sci.*, **33**, 315-325.
- Toyoshima, S., Kanno, A., Kitayama, T., Sekiya, K., Nakai, K., Haruna, M., Mino, T., Miyazaki, H., Yano, K. and Yamamoto, K. (2005): QT PRODACT: *in vivo* QT assay in the conscious dog for assessing the potential for QT interval prolongation by human pharmaceuticals. *J. Pharmacol. Sci.*, **99**, 459-471.
- van der Linde, H.J., Van Deuren, B., Teisman, A., Towart, R. and Gallacher, D.J. (2008): The effect of changes in core body temperature on the QT interval in beagle dogs: a previously ignored phenomenon, with a method for correction. *Br. J. Pharmacol.*, **154**, 1474-1481.
- Van Deuren, B., Van Ammel, K., Somers, Y., Cools, F., Straetmans, R., van der Linde, H.J. and Gallacher, D.J. (2009): The fentanyl/etomidate-anaesthetised beagle (FEAB) dog: a versatile *in vivo* model in cardiovascular safety research. *J. Pharmacol. Toxicol. Methods*, **60**, 11-23.
- Webster, R., Allan, G., Anto-Awuakye, K., Harrison, A., Kidd, T., Leishman, D., Phipps, J. and Walker, D. (2001): Pharmacokinetic/pharmacodynamic assessment of the effects of E4031, cisapride, terfenadine and terodiline on monophasic action potential duration in dog. *Xenobiotica*, **31**, 633-650.
- Zhou, Z., Vorperian, V.R., Gong, Q., Zhang, S. and January, C.T. (1999): Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole. *J. Cardiovasc. Electrophysiol.*, **10**, 836-843.